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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/82, 15/54, 9/10, A01H 5/00 . A2

(11) International Publication Number: **WO 00/08190**

(43) International Publication Date: 17 February 2000 (17.02.00)

(21) International Application Number: PCT/US99/17456

(22) International Filing Date: 2 August 1999 (02.08.99)

(30) Priority Data:
09/128,339 3 August 1998 (03.08.98) US

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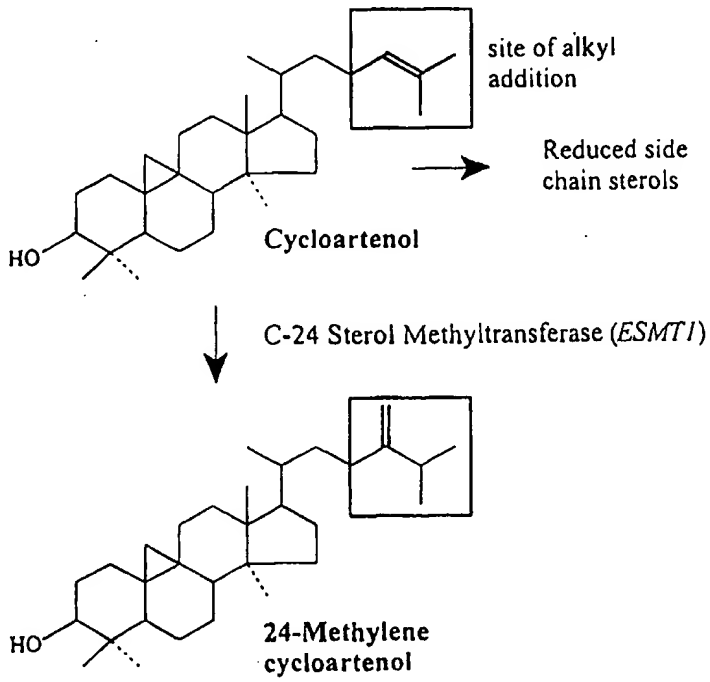
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: STEROL METHYLTRANSFERASE GENE

(57) Abstract

Disclosed is an isolated DNA sequence that encodes a *Zea mays* C-24 sterol methyltransferase. A yeast *erg6* transformant containing the C-24 sterol methyltransferase gene produces ergosterol, whereas untransformed isolates of yeast *erg6* do not produce ergosterol. Also disclosed is a method of altering sterol metabolism in a plant by transforming the plant with a heterologous construct comprising a *Z. mays* C-24 methyltransferase coding sequence operably connected to a plant promoter. Plants having altered sterol metabolism are expected to exhibit unique phenotypes, including reduced ability to support feeding pests that depend on plant sterols for completion of their life cycles.



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STEROL METHYLTRANSFERASE GENE

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not applicable.

BACKGROUND OF THE INVENTION

Each year, insects, nematodes, and fungi that feed on plants contribute significantly to reduced agricultural productivity and profitability. Traditionally, control of infestations of food crops by plant pests has been achieved through the use of pesticides. It is now recognized that the use of such chemicals can adversely affect the environment, the ecology, and human health.

The topical application of pesticides to crops is problematic because it is virtually impossible to effectively target specific fields and specific organisms. Most pesticides exhibit toxicity toward a relatively broad range of organisms, and are effective against species that are not considered to be undesirable, or which are regarded as beneficial.

Considerable research has been directed toward developing alternative means of protecting plants against plant pests. Increasingly, plant molecular biologists are seeking to develop genetically engineered plant strains that have desirable characteristics, such as increased resistance to pests. One means by which resistance can be engineered is to alter plant metabolism such that the plant does not provide the feeding pest with nutrients required for completion of its life cycle.

Sterols are a class of lipids that have been found to be essential for the synthesis and maintenance of membranes in

most eukaryotic organisms studied. Insects, nematodes, and some species of fungi are unable to synthesize sterols and are dependent on plants for the sterols required for the completion of their life cycles. More than 250 different sterols are produced by plants. Certain plant species produce as many as 60 different sterols.

What is needed in the art is a means of altering sterol metabolism in plants in order to control populations of feeding plant pests.

BRIEF SUMMARY OF THE INVENTION

The present invention is an isolated DNA sequence that is substantially homologous to the *Zea mays* C-24 sterol methyltransferase coding sequence shown in SEQ ID NO:1. The amino acid sequence encoded by the open reading frame (ORF) is shown in SEQ ID NO:2.

The present invention is also a heterologous genetic construct comprising a DNA sequence that is substantially homologous to the *Zea mays* C-24 sterol methyltransferase coding sequence shown in SEQ ID NO:1 operably connected to a promoter that promotes gene expression in plants.

Another aspect of the present invention is a method of altering sterol metabolism comprising the steps of: (a) providing a heterologous genetic construct comprising a DNA sequence that encodes a C-24 sterol methyltransferase having at least 80% amino acid identity with SEQ ID NO:2 operably connected to a promoter that promotes gene expression in plants; and (b) introducing the genetic construct into a plant.

In another embodiment, the present invention is a plant comprising in its genome a genetic construct comprising a DNA sequence encoding a C-24 sterol methyltransferase operably connected to a promoter that promotes gene expression in plants.

It is an object of the present invention to provide a DNA sequence encoding a sterol methyltransferase, which when expressed in plants, alters sterol metabolism in the plant so as to afford protection against infestation by pests to which

the plant is ordinarily susceptible.

It is an object of the present invention to provide a method of protecting plants against pest infestation.

It is an object of the present invention to provide a pest resistant plant.

Other objects, features, and advantages of this invention will become apparent upon review of the specification and claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1 shows the proposed methylation of cycloartenol by a *Zea mays* endosperm C-24 sterol methyltransferase.

Figure 2 shows a comparison between the deduced amino acid sequences of maize ESMT1 (SEQ ID NO:2) and C-24 sterol methyltransferase cDNA sequences from *Arabidopsis*, yeast, and soybean.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, the present invention is an isolated DNA fragment comprising a sequence that is substantially homologous to a *Zea mays* endosperm C-24 sterol methyltransferase (ESMT1) coding sequence (SEQ ID NO:1).

The methyltransferase of this invention catalyzes the alkylation at carbon 24 of sterol intermediates. In higher plants, the sterols that contain C-24 alkyl additions predominate, whereas unmethylated sterols such as cholesterol are present at low levels. The production of alkylated sterols occurs after cyclization of squalene and involves several stages predicated on the alkylation of the double bond at carbon 24 in the sterol side chain in a reaction catalyzed by an S-adenosyl-L-methionine methyltransferase (SMT) (Benveniste, Annu Rev Plant Physiol 37:275-308, 1986). Alkylation at carbon 24 by sterol methyltransferase is believed to be a key step in the regulation of carbon flux through the sterol biosynthetic pathway (Beneviste, *supra*; Chappell *et al*. Plant Physiol 109:1337-1343, 1995). A proposed reaction catalyzed by ESMT1 in maize is shown in Fig. 1.

Sterol methyltransferase cDNA clones have been obtained from *Arabidopsis thaliana* (Husselstein *et al.* FEBS Lett. 381:87-92, 1996) and soybean (Shi *et al.*, J. Biol. Chem. 271:9384-9399, 1996). The putative proteins encoded by these clones were found to have homology to the yeast ERG6 protein. The expression of the cloned *Arabidopsis* sterol methyl-transferase (SMT) gene in *erg6* yeast was found to complement the *erg6* deficiency by allowing low level production of ergosterol, and it was discovered that the enzyme catalyzes a second methyl transfer that results in the formation of large amounts of C-24 ethyl sterols.

We have isolated and characterized a C-24 methyl-transferase DNA coding sequence of the present invention from *Z. mays* endosperm as described in the examples below and in Grebenok, et al. (Plant Mol. Biol. 34:891-896, 1997), which is incorporated by reference herein. Briefly, a *Z. mays* endosperm cDNA (53D6) was selected from an EST database based on its sequence identity with the amino terminal portion of the yeast ERG6 protein. A clone containing the cDNA as a *Not*I insert in the plasmid λZap was then obtained from Pioneer Hybrid. The DNA sequence of the cDNA is shown in SEQ ID NO:1. It would be well within the ability of one skilled in the art to obtain other maize sterol methyl-transferase DNA coding sequences using known methods. For example, one wishing to obtain a C-24 sterol methyltransferase DNA coding sequence could screen a genomic or cDNA library from any plant with a probe complementary to a portion of the coding region of SEQ ID NO:1.

A *Zea mays* endosperm sterol methyltransferase coding sequence is any DNA sequence that has substantial homology to SEQ ID NO:1. By "substantial homology" it is meant a DNA sequence that encodes a protein that has at least 80% amino acid identity with SEQ ID NO:2, and which exhibits C-24 sterol methyltransferase activity. Preferably, the DNA sequence encodes a protein that has an amino acid identity with SEQ ID NO:2 of at least 90%. Most preferably, the DNA sequence encodes a polypeptide that has an amino acid identity with SEQ ID NO: 2 of about 95% or higher.

A putative C-24 sterol methyltransferase coding sequence could be confirmed by evaluating the activity of a C-24 sterol methyltransferase gene product by yeast *erg6* complementation as described below in the examples. When expressed in an *erg6* strain of *Saccharomyces cerevisiae*, C-24 sterol methyltransferase allows survival of the yeast on cyclohexamide, presumably by catalyzing a methyl addition to a sterol intermediate to form ergosterol. A putative C-24 sterol methyltransferase coding sequence could be confirmed by expressing the gene in a suitable expression system and evaluating the ability of the gene product to methylate an appropriate substrate, such as cycloartenol, lanosterol, or zymosterol (Nes *et al. supra*).

The C-24 sterol methyltransferase of the present invention is distinguishable from the *Arabidopsis* SMT by its inability to catalyze the transfer of a second methyl group to a 24-methyl sterol to form a 24-ethyl sterol. It is expected that the substrate specificity of the C-24 sterol methyltransferase of the present invention may be altered by introducing regions of the *Arabidopsis* SMT gene or other sterol methyltransferase genes that encode sterol methyltransferases having different substrate specificities into a sequence comprising SEQ ID NO:1.

It is expected that polyploid plants having more than one copy of the C-24 sterol methyltransferase gene may have allelic variations among C-24 sterol methyltransferase gene sequences. It is anticipated that putative C-24 sterol methyltransferase sequences having less than 100% sequence identity to SEQ ID NO:1 encode proteins having sterol methyltransferase activity that are encompassed by the sequence of the present invention.

It is envisioned that minor sequence variations from SEQ ID NO:1 associated with nucleotide additions, deletions, and mutations, whether naturally occurring or introduced in vitro, will not affect C-24 sterol methyltransferase activity. The scope of the present invention is intended to encompass minor variations in C-24 sterol methyltransferase sequences.

It is also envisioned that many DNA sequences can be used to code for the expression of a single protein. For example,

using codon substitution it is known that there are many DNA sequences other than SEQ ID No: 1 which will encode the protein of SEQ ID No: 2. There are also known to be conservative amino acid substitutions that can be made, particularly in portions of the protein not at critical catalytic sites, which are highly unlikely to change protein function. It is intended that the sequence of the present specification be interpreted to encompass such variations.

The expression of C-24 sterol methyltransferase in a yeast *erg6* background allows the formation of ergosterol, which is absent in *erg6* yeast strains. In the examples below, the relative amount of ergosterol produced in *erg6* yeast transformed with the C-24 sterol methyltransferase coding sequence was about 10% of that produced in wild-type yeast. It is expected that a C-24 sterol methyltransferase which when expressed in *erg6* yeast results in ergosterol production at a level that is higher or lower than 10% of wild-type ergosterol production would be suitable in the practice of the present invention.

The C-24 sterol methyltransferase sequence that we identified is a cDNA from *Z. mays* endosperm (ESMT1) encoding a protein with homology to C-24 sterol methyltransferases from soybean (Shi et al., *supra*), *Arabidopsis* (Husselstein et al., *supra*) and yeast (Garber et al. Mol. Cell Biol. 9:3447-3456, 1989). Analysis of the deduced amino acid sequence of ESMT1 revealed several conserved motifs (Figure 1), three of which are found in a large number of S-adenosyl-L-methionine-dependent methyltransferases and are thought to contribute to the binding of S-adenosyl-methionine (Kagen and Clarke Arch Biochem Biophys 310:417-427, 1994).

In addition to the conserved SAM motifs in ESMT1, sequence alignment of corn, *Arabidopsis*, and soybean SMTs and yeast ERG6 protein reveals two other highly conserved regions, designated SMT I and SMT II, which are unique to the SMTs. We have suggested that the SMTs may define the active site and/or substrate binding sites (Grebenok et al., Plant Mol. Biol. 34:891-896, 1997). Sequence alignments also identified at

least two additional regions, A and B, which share a high level of homology between maize, soybean and yeast but show significant divergence in the *Arabidopsis* SMT. Motifs A and B could define functional sites within the *A. thaliana* SMT that are necessary for multiple methyl additions. We have proposed that two classes of sterol methyltransferase may exist in plants, one defined by ESMT1 and the soybean SMT, and a second defined by the *Arabidopsis* SMT (Grebenok et al., *supra*).

Higher plant sterol methyltransferases have been shown to exhibit substrate specificity with respect to side chain conformation. Nes *et al.* demonstrated that a side chain stereochemistry identical to that of cycloartenol was necessary for attaining maximal enzyme activity in *in vitro* enzyme assays with purified sunflower SMT (J. Biol. Chem 266:15202-15212, 1991). The precursors zymosterol, which is a precursor for C-24 methylation in yeast, and lanosterol were used less efficiently as substrates by plant enzymes *in vitro*, with reaction velocities of about 30% of maximum SMT activity observed for the plant substrate cycloartenol.

The low level ergosterol production (10% wild-type) found in the complementation of the yeast *erg6* mutant by maize ESMT1 may be due to a lower efficiency in using the zymosterol substrate, as reported for the isolated enzyme (Nes *et al.*, *supra*). Alternatively, in view of the localization of sterol biosynthetic activities to microsomal fractions in yeast and higher plants (Moore and Gaylor, J. Biol. Chem. 244:6334-6340, 1969), the sub-wild-type level of functional complementation with ESMT1 may reflect inappropriate targeting or failure of the enzyme to interact efficiently with the yeast sterol biosynthetic machinery.

Construction of an expression vector comprising a *Zea mays* C-24 sterol methyltransferase coding sequence operably connected to a plant promoter not natively associated with the coding sequence is planned using standard molecular biology techniques known to the art. The plant promoter may be any plant promoter, including a constitutive promoter such as CaMV 35S, which is known to function in a wide variety of plants.

Other promoters that are functional in plants may be used to create the genetic constructs to be used in the practice of this invention. These may include other constitutive promoters, tissue-specific promoters, developmental stage-specific promoters, and inducible promoters. Promoters may also contain certain enhancer sequence elements that improve the efficiency of transcription. Optionally, the construct may contain a termination signal, such as the nopaline synthase terminator (NOS). Preferably, the constructs will include a selectable or screenable marker to facilitate identification of transformants. The constructs may have the coding region in the sense or antisense orientation.

Once a genetic construct comprising a C-24 sterol methyl-transferase gene has been obtained, it can readily be introduced into a plant or plant tissue using standard methods known to the art. For example, the *Agrobacterium* transformation system is known to work well with all dicot plants and some monocots. Other methods of transformation equally useful in dicots and monocots may also be used. Transgenic plants may be obtained by particle bombardment, electroporation, or by any other method of transforming plants known to one skilled in the art of plant molecular biology. The experience to date in the technology of plant genetic engineering has taught that the method of gene introduction does not affect the phenotype achieved in the transgenic plants.

A transgenic plant may be obtained directly by transformation of a plant cell in culture, followed by regeneration of a plant. Also, transgenic plants may be obtained from transgenic seeds set by parental transgenic plants. Transgenic plants pass on inserted genes, sometimes referred to as transgenes, to their progeny by normal Mendellian inheritance just as they do their native genes. Methods for breeding and regenerating plants of agronomic interest are known in the art.

It is reasonable to expect that the expression of heterologous C-24 sterol methyltransferase in a transgenic plant will result in alterations in the sterol profile in that

plant. Changes in the sterol profile can be expected to result in unique, advantageous phenotypes, including the reduced ability to support a feeding pest that depends on plant sterols for completion of its life cycle. This invention is intended to encompass other advantageous phenotypes in addition to interfering with the life cycle of feeding pests that may result from alterations in sterol metabolism in plants obtained by the practice of this invention.

The following nonlimiting examples are intended to be purely illustrative.

Examples

Strains

Saccharomyces cerevisiae strain *erg6* (α *leu2 ura3* *erg6*::LEU2) and the corresponding wild type yeast strain were kindly provided by L. Parks (North Carolina State University). The *Z. mays* endosperm cDNA 53D6, contained as a Not*I* insert within plasmid λZAP(Strategene, LaJolla, CA), was provided by T. Helentjaris (Pioneer HiBred).

Growth and transformation conditions

The *erg6* mutant and wild type were grown YPD (1% yeast extract (Difco), 2% bactopeptone (Difco), 2% dextrose). Electrocompetent yeast was prepared, electroplated and plated according to the method of Becker and Guarente (Meth. Enzymol 194:182-187, 1991). Yeast strain *erg6* transformants were selected on complete synthetic media without uracil (0.67% yeast nitrogen base without amino acids (Difco) and 2% galactose). E. coli strain DH5α was used for routine cloning according to standard, established procedures.

Identification and Cloning of *Zea mays* C-24 sterol methyltransferase

A *Z. mays* endosperm cDNA was selected from an EST database based on its sequence identity with the amino terminal portion of the yeast ERG6 protein. The cDNA insert from plasmid 53D6 was excised with *Kpn*I-*Hind*III and ligated into a linearized pBluescript II vector (Stratagene) having compatible ends to form pRJG3. The cDNA insert from plasmid pRJG3 was excised using *Eco*RI and was ligated into the similarly cut yeast expression vector λYES (Elledge et al. Proc Natl Acad Sci USA 88:1731-1735, 1991). To form pRGJ6. Plasmid DNA was isolated from complemented yeast using previously reported procedures (Hoffman and Winston, Gene 57:267-272, 1987). Automated DNA sequencing was performed at the Arizona Biotechnology Facility after transfer of the *Not*I/*Hind*III fragment of 53D6 into pBluescript II (Stratagene). The full-length cDNA was sequenced and found to contain an open reading frame of 1.5 kb encoding a 40 kDa protein with 46% identity to the yeast ERG6 protein (Figure 2).

The predicted amino acid sequence of ESMT1 shows 66% similarity and 46% identity to the *S. Cerevisiae* ERG6 protein and 75% and 37% identity to the soybean and *Arabidopsis* SMTs, respectively (Table 1). Alignment of all four methyltransferase cDNAs yields a shared identity of 36%, while alignment of the soybean, maize and yeast methyltransferase cDNA sequences without addition of the *Arabidopsis* cDNA sequence yields 44% identity. Hydropathy analysis indicates the presence of a 25 amino acid leader peptide on the *A. thaliana* protein but no on the *Z. mays*, soybean or yeast SMTs (results not shown). Analysis of the ESMT1 protein sequence indicated the presence of three conserved motifs comparable in sequence, length, order and spacing to those identified in diverse S-adenosyl-L-methionine-dependant methyltransferases described by Kagan and Clarke (Arch Biochem Biohpys 310:417-427, 1994)(Figure 2). The presence of these motifs support identification of *Z. mays* ESMT1 as an S-adenosyl-methionine-dependant methyltransferase. These three SAM motifs are also conserved in sequence, order, length and spacing in the yeast, soybean and *Arabidopsis* SMTs (Figure 2). Alignment of all four SMT sequences identifies two additional highly conserved regions. SMT motif I (SMT I) is 11 amino acids long, begins with the conserved aromatic amino acid phenylalanine found at position 65 of the *Z. mays* sequence and contains 9 amino acids identical in all 4 proteins. SMT motif II (SMT II) begins directly after the SAM II site, Glu-179, and consists of 8 contiguous amino acids which are identical in all 4 proteins. These two SMT motifs, possibly acting in concert may represent an active site (sterol-binding site) for higher plant SMTs. Two additional regions (motifs A and B) were identified based upon sequence identity in the soybean, *Z. mays* and ERG6 SMTs but not in the *Arabidopsis* SMT (Figure 2). Motif A is located directly upstream of the SAM II site beginning with phenylalanine 157 and spans 9 amino acids, 8 of which are identical within maize, soybean, and yeast, while only 3 are conserved in *A. thaliana* Motif B, located very near the carboxy end of the proteins, begins with leucine 304 and spans 13 amino

acids, 10 of which are identical within the maize, soybean and yeast sequences, while only 5 are conserved in *Arabidopsis* (Figure 2).

Complementation of yeast *erg6*

Unlike wild-type yeast, the *erg6* strain exhibits sensitivity to cycloheximide, an inhibitor of protein synthesis. Cycloheximide sensitivity is due altered cell membrane permeability that is a consequence of the inability of this strain to produce ergosterol. The *Z. mays* endosperm cDNA was initially characterized based on its ability to relieve cycloheximide sensitivity in *erg6*. The yeast strain *erg6* was transformed with the isolated *Z. mays* endosperm cDNA contained in a yeast expression vector under the transcriptional control of the GAL4 promoter (pRJG6) by electroporation. Transformants were cultured for 4 days at 30°C on complete synthetic minimal media lacking uracil and with 5% galactose as the sole carbon source to induce activity of the GAL4 promoter. Colonies were subsequently replica plated onto fresh complete synthetic media lacking uracil but containing cycloheximide (0.1 μg/ml). After further incubation for 3 days at 30°C, individual colonies demonstrating improved or wild-type growth were selected for sterol analysis.

A transformation efficiency of 2 x 10^2 colony-forming units per μg DNA was observed. Complementing the mutant phenotype is a consequence of the production of ergosterol, which restores the wild-type permeability characteristic of the plasma membrane and leads to exclusion of cycloheximide from the cytoplasm (Garber *et al.* Mol. Cell. Biol. 9:3447-3456, 1989). Depending on the level of ergosterol production, complemented cells exhibit a percentage of wild-type growth in the presence of cycloheximide, whereas non-complemented cells remain sensitive. Without exception, the *erg6* mutant cells containing the 53D6 cDNA in λYES (pRJG6) were able to grow under cycloheximide selection in the presence of galactose, but demonstrated cycloheximide sensitivity in the absence of galactose.

Sterol analysis

Those colonies selected for sterol analysis were cultured in complete synthetic liquid media lacking uracil and supplemented with cycloheximide (0.1 μg/ml). Cells from 50 ml aliquots were collected by centrifugation at 2000 rpm for 10 min. The supernatant was discarded and the pellets were extracted for 12 h in 50 ml methanol. The methanol fraction was removed and evaporated to dryness in a rotary evaporator. Sterols were isolated according to Garber et al. (Mol Cell Biol 9:3447-3456, 1989), and were identified by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard 5890 gas chromatograph and a Hewlett-Packard 5970 mass spectrometer. GC separation was carried out on a HP-1 column (15m x .3mm) with 0.25μm film thickness. Column temperature was programmed from 120 to 300°C at 4°C/min and the carrier gas was helium at a velocity of 30 cm/s. The MS was operated at an ionizing potential of 100 eV, and the ion source was maintained at 300°C. Ergosterol produced in the complemented *erg6* yeast cells was identified through co-chromatography with authentic ergosterol on the gas chromatograph, and by a mass spectrum with characteristic ions at m/e: 396 [M+], 363, 337, 271, 253, and 211 (Rahier and Benveniste, "Mass spectral identification of phytosterols." *In*: New WD, Parish EF (eds) Analysis of Sterol and other Biologically Significant Steroids, pp 223-250, Academic Press Publishers, New York, 1989).

Sterols were isolated and characterized from both the *erg6* mutant and the complemented mutant lines. When propagated in complex synthetic liquid medium the *erg6* mutant line produced cholesta-8,24-dien-3β-ol (M+384) which accounted for 72% and 20% of the isolated sterol, respectively. Two minor sterols, each representing 4% of the isolated sterol, had molecular weights of 382, suggesting cholesta-triene-3β-ol structure and the final sterol representing 1% of the isolated sterol is presumably the cholesta-tetraene-3β-ol (M+380). Based upon molecular weights, none of the aforementioned sterols contained methyl additions to their side chains accounting for the cycloheximide sensitivity observed in the *erg6* mutant

(Husselstein *et al.* FEBS lett 381:87-92, 1996).

When propagated in complete synthetic liquid medium in the presence of galactose and cycloheximide, the transformed colonies produced in addition to the previously mentioned sterols, two new peaks, one of which comigrated with purified ergosterol (results not shown). The molecular weights of the new sterols were 396 and 394, respectively. The sterol with a molecular weight of 394 is presumably a ergostatetraene (Husselstein *et al., supra*). Ergosterol production reached a maximum level of about 5% of the total isolated sterol pool, while in wild-type yeast, ergosterol normally comprises about 45% of the sterol pool (Garber, *et al.* Mol Cell Biol 9:3447-3456, 1996). The level of ergosterol produced within the transformants, although about 10% of wild type, accounts for the cycloheximide resistance observed (Garber *et al. supra;* Husselstein *et al., supra*).

The present invention is not limited to the exemplified embodiments, but is intended to encompass all such modifications and variations as come within the scope of the following claims.

CLAIM OR CLAIMS

WE CLAIM:

1. An isolated DNA fragment encoding a C-24 sterol methyltransferase from maize comprising a sequence that is substantially homologous to SEQ ID NO:1.

2. The fragment of claim 1, wherein the deduced amino acid sequence of the coding sequence has at least 80% amino acid identity with SEQ ID NO:2.

3. The fragment of claim 2, wherein the amino acid sequence has at least 90% identity with the sequence of SEQ ID NO:2.

4. The fragment of claim 2, wherein the amino acid sequence has at least 95% identity with the sequence of SEQ ID NO:2.

5. The fragment of claim 1, wherein the coding sequence comprises SEQ ID NO:1.

6. A genetic construct comprising the coding sequence of claim 1 operably connected to a plant promoter not natively associated with the coding sequence.

7. The genetic construct of claim 6, wherein the coding sequence comprises SEQ ID NO:1.

8. A method of obtaining a plant comprising in its genome a DNA sequence encoding a heterologous C-24 sterol methyltransferase from maize comprising the steps of:

a) making a genetic construct comprising a DNA sequence encoding a C-24 sterol methyltransferase having the SEQ ID NO:2 operably connected to a promoter functional in plants; and

b) introducing the genetic construct into a plant cell; and

c) regenerating a plant from the plant cell of step b.

9. The method of claim 8, wherein the genetic construct of step a comprises SEQ ID NO:1.

10. A plant comprising in its genome the genetic construct of claim 6.

11. A seed of the plant of claim 10.

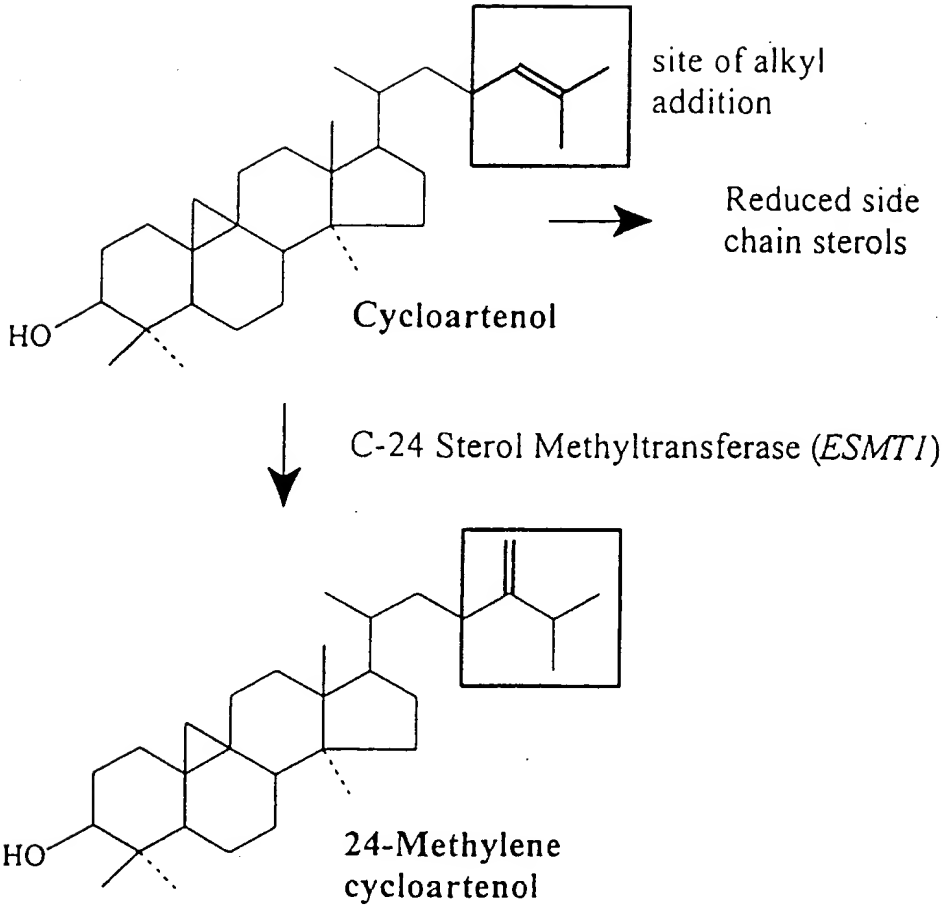
site of alkyl addition
Reduced side chain sterols
HO
Cycloartenol
C-24 Sterol Methyltransferase (*ESMT1*)
HO
24-Methylene cycloartenol

FIG 1

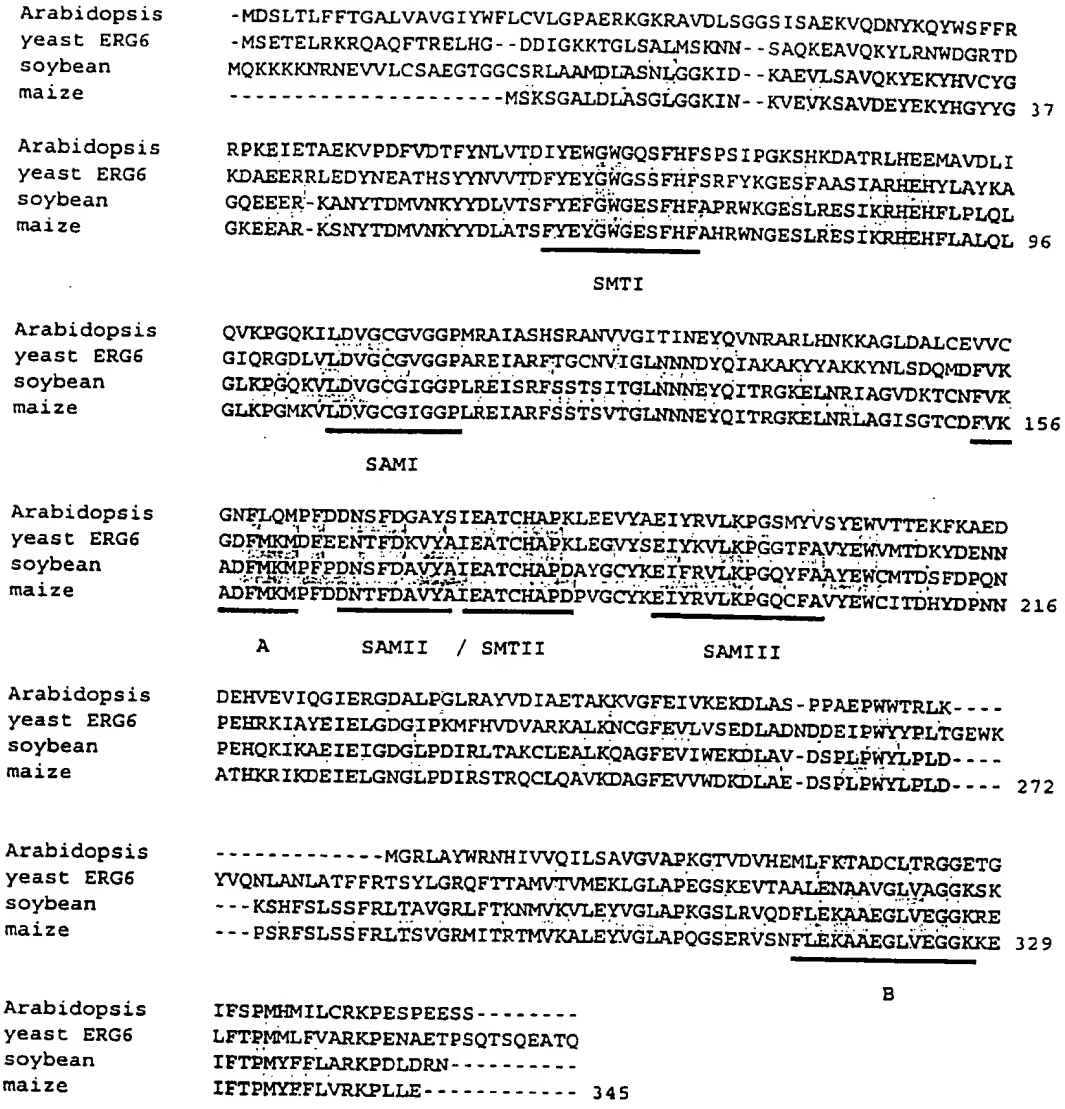
Arabidopsis -MDSLTLFFTGALVAVGIYWFLCVLGPAERKGKRAVDLSGGSISAEKVQDNYKQYWSFFR
yeast ERG6 -MSETELRKRQAQFTRELHG--DDIGKKTGLSALMSKNN--SAQKEAVQKYLRNWDGRTD
soybean MQKKKKNRNEVVLCSAEGTGGCSRLAAMDLASNLGGKID--KAEVLSAVQKYEKYHVCYG
maize ----------MSKSGALDLASGLGGKIN--KVEVKSAVDEYEKYHGYYG 37
Arabidopsis RPKEIETAEKVPDFVDTFYNLVTDIYEWGWGQSFHFSPSIPGKSHKDATRLHEEMAVDLI
yeast ERG6 KDAEERRLEDYNEATHSYYNVVTDFYEYGWGSSFHFSRFYKGESFAASIARHEHYLAYKA
soybean GQEEEER-KANYTDMVNKYYDLVTSFYEFGWGESFHFAPRWKGESLRESIKRHEHFLPLQL
maize GKEEAR-KSNYTDMVNKYYDLATSFYEYGWGESFHFAHRWNGESLRESIKRHEHFLALQL 96
SMTI
Arabidopsis QVKPGQKILDVGCGVGGPMRAIASHSRANVVGITINEYQVNRARLHNKKAGLDALCEVVC
yeast ERG6 GIQRGDLVLDVGCGVGGPAREIARFTGCNVIGLNNNDYQIAKAKYYAKKYNLSDQMDFVK
soybean GLKPGQKVLDVGCGIGGPLREISRFSSTSITGLNNNEYQITRGKELNRIAGVDKTCNFVK
maize GLKPGMKVLDVGCGIGGPLREIARFSSTSVTGLNNNEYQITRGKELNRLAGISGTCDFVK 156
SAMI
Arabidopsis GNFLQMPFDDNSFDGAYSIEATCHAPKLEEVYAEIYRVLKPGSMYVSYEWVTTEKFKAED
yeast ERG6 GDFMKMDFEENTFDKVYAIEATCHAPKLEGVYSEIYKVLKPGGTFAVYEWVMTDKYDENN
soybean ADFMKMPFPDNSFDAVYAIEATCHAPDAYGCYKEIFRVLKPGQYFAAYEWCMTDSFDPQN
maize ADFMKMPFDDNTFDAVYAIEATCHAPDPVGCYKEIYRVLKPGQCFAVYEWCITDHYDPNN 216
A SAMII / SMTII SAMIII
Arabidopsis DEHVEVIQGIERGDALPGLRAYVDIAETAKKVGFEIVKEKDLAS-PPAEPWWTRLK----
yeast ERG6 PEHRKIAYEIELGDGIPKMFHVDVARKALKNCGFEVLVSEDLADNDDEIPWYYPLTGEWK
soybean PEHQKIKAEIEIGDGLPDIRLTAKCLEALKQAGFEVIWEKDLAV-DSPLPWYLPLD----
maize ATHKRIKDEIELGNGLPDIRSTRQCLQAVKDAGFEVVWDKDLAE-DSPLPWYLPLD---- 272
Arabidopsis ----------MGRLAYWRNHIVVQILSAVGVAPKGTVDVHEMLFKTADCLTRGGETG
yeast ERG6 YVQNLANLATFFRTSYLGRQFTTAMVTVMEKLGLAPEGSKEVTAALENAAVGLVAGGKSK
soybean ---KSHFSLSSFRLTAVGRLFTKNMVKVLEYVGLAPKGSLRVQDFLEKAAEGLVEGGKRE
maize ---PSRFSLSSFRLTSVGRMITRTMVKALEYVGLAPQGSERVSNFLEKAAEGLVEGGKKE 329
B
Arabidopsis IFSPMHMILCRKPESPEESS--------
yeast ERG6 LFTPMMLFVARKPENAETPSQTSQEATQ
soybean IFTPMYFFLARKPDLDRN----------
maize IFTPMYFFLVRKPLLE---------- 345

FIG 2

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Grebenok, Robert J.
Galbraith, David W.
DellaPenna, Dean

(ii) TITLE OF INVENTION: STEROL METHYLTRANSFERASE GENE

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Quarles & Brady

(B) STREET: 1 South Pinckney Street

(C) CITY: Madison

(D) STATE: WI

(E) COUNTRY: US

(F) ZIP: 53701-2113

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US

(B) FILING DATE:

(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Seay, Nicholas J.

(B) REGISTRATION NUMBER: 27,386

(C) REFERENCE/DOCKET NUMBER: 920214.90166

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (608)251-5000

(B) TELEFAX: (608)251-9166

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1383 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 78..1112

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CCCACGCGTC CGAGACTCTG GTTCTGACAT GCAGCAATTA TTGCAGGTGC ATTTGATCCG      60

TCCCGGCCGC CTACACG ATG TCC AAG TCG GGA GCG CTG GAT CTT GCT TCT         110
                   Met Ser Lys Ser Gly Ala Leu Asp Leu Ala Ser
                     1               5                  10

GGC CTC GGA GGG AAG ATC AAC AAG GTG GAA GTC AAG TCG GCC GTC GAT        158
Gly Leu Gly Gly Lys Ile Asn Lys Val Glu Val Lys Ser Ala Val Asp
             15                  20                  25

GAG TAT GAG AAA TAT CAT GGA TAC TAT GGA GGG AAG GAG GAA GCA AGG        206
Glu Tyr Glu Lys Tyr His Gly Tyr Tyr Gly Gly Lys Glu Glu Ala Arg
         30                  35                  40

AAG TCC AAC TAT ACT GAT ATG GTT AAT AAA TAC TAT GAT CTT GCC ACT        254
Lys Ser Asn Tyr Thr Asp Met Val Asn Lys Tyr Tyr Asp Leu Ala Thr
     45                  50                  55

AGC TTC TAT GAG TAT GGT TGG GGT GAA TCC TTC CAC TTT GCT CAC AGA        302
Ser Phe Tyr Glu Tyr Gly Trp Gly Glu Ser Phe His Phe Ala His Arg
 60                  65                  70                  75

TGG AAT GGA GAA TCC TTA CGT GAA AGC ATC AAG CGA CAT GAG CAT TTT        350
Trp Asn Gly Glu Ser Leu Arg Glu Ser Ile Lys Arg His Glu His Phe
                 80                  85                  90

CTT GCC CTG CAA CTT GGT TTG AAA CCA GGA ATG AAG GTT TTA GAT GTG        398
Leu Ala Leu Gln Leu Gly Leu Lys Pro Gly Met Lys Val Leu Asp Val
             95                 100                 105

GGC TGT GGA ATA GGT GGA CCA CTG AGA GAA ATT GCA AGA TTT AGC TCA        446
Gly Cys Gly Ile Gly Gly Pro Leu Arg Glu Ile Ala Arg Phe Ser Ser
         110                 115                 120

ACT TCA GTT ACC GGA TTG AAT AAC AAC GAA TAC CAG ATA ACC AGG GGA        494
Thr Ser Val Thr Gly Leu Asn Asn Asn Glu Tyr Gln Ile Thr Arg Gly
     125                 130                 135

AAG GAG CTC AAC CGT TTA GCA GGA ATT AGT GGA ACA TGT GAT TTT GTC        542
Lys Glu Leu Asn Arg Leu Ala Gly Ile Ser Gly Thr Cys Asp Phe Val
140                 145                 150                 155
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AAG GCG GAC TTC ATG AAG ATG CCG TTC GAT GAC AAC ACT TTT GAT GCT      590
Lys Ala Asp Phe Met Lys Met Pro Phe Asp Asp Asn Thr Phe Asp Ala
                160                 165                 170

GTT TAC GCC ATT GAG GCA ACA TGT CAT GCA CCT GAT CCA GTT GGT TGC      638
Val Tyr Ala Ile Glu Ala Thr Cys His Ala Pro Asp Pro Val Gly Cys
            175                 180                 185

TAC AAG GAG ATA TAT CGT GTG TTG AAG CCT GGC CAG TGC TTT GCC GTG      686
Tyr Lys Glu Ile Tyr Arg Val Leu Lys Pro Gly Gln Cys Phe Ala Val
        190                 195                 200

TAC GAG TGG TGC ATT ACG GAT CAC TAT GAT CCT AAC AAT GCA ACC CAC      734
Tyr Glu Trp Cys Ile Thr Asp His Tyr Asp Pro Asn Asn Ala Thr His
    205                 210                 215

AAA AGG ATC AAG GAT GAA ATT GAG CTT GGC AAT GGC CTG CCA GAT ATC      782
Lys Arg Ile Lys Asp Glu Ile Glu Leu Gly Asn Gly Leu Pro Asp Ile
220                 225                 230                 235

AGA AGC ACT CGG CAA TGT CTC CAG GCA GTA AAA GAC GCC GGG TTT GAG      830
Arg Ser Thr Arg Gln Cys Leu Gln Ala Val Lys Asp Ala Gly Phe Glu
                240                 245                 250

GTT GTT TGG GAT AAG GAT CTT GCT GAA GAT TCT CCC TTG CCT TGG TAC      878
Val Val Trp Asp Lys Asp Leu Ala Glu Asp Ser Pro Leu Pro Trp Tyr
            255                 260                 265

TTG CCC TTG GAT CCA AGC CGA TTC TCC CTG AGT AGC TTC CGT TTG ACC      926
Leu Pro Leu Asp Pro Ser Arg Phe Ser Leu Ser Ser Phe Arg Leu Thr
        270                 275                 280

TCT GTG GGA CGC ATG ATT ACC CGC ACA ATG GTC AAG GCC CTG GAG TAC      974
Ser Val Gly Arg Met Ile Thr Arg Thr Met Val Lys Ala Leu Glu Tyr
    285                 290                 295

GTT GGT CTT GCT CCG CAG GGC AGT GAG AGG GTC TCT AAT TTC CTG GAG      1022
Val Gly Leu Ala Pro Gln Gly Ser Glu Arg Val Ser Asn Phe Leu Glu
300                 305                 310                 315

AAG GCT GCA GAA GGG CTG GTA GAG GGC GGA AAG AAG GAG ATC TTC ACG      1070
Lys Ala Ala Glu Gly Leu Val Glu Gly Gly Lys Lys Glu Ile Phe Thr
                320                 325                 330
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CCA ATG TAC TTC TTT CTT GTT CGG AAG CCT CTT CTG GAA TGA 1112
Pro Met Tyr Phe Phe Leu Val Arg Lys Pro Leu Leu Glu *
335 340 345

GCTCTTGGAT CACCTTTTCA GAGAGAGAAG GCAAGTGGTC ATTTCGAAGA AGCCGAGGAG 1172

AGGGAACCTG GAATCAAGAA AACCTTCAGC TCTCCTGTGT AGGAGGAAAG TTAACGAACA 1232

GTGTAGTAAC TGTTCAGCTT TGTGTTTATT CAGTTGTTTT GCTGCTTGAG GTTATTCGTT 1292

TCTAGGTGGG GGTTGGAATC CTTTTCGCCA TAAACCTCTC AGTGGCATAA ATAAGATGGT 1352

TTGCATAAAA AAAAAAAAAA AAAAAAAAAA A 1383

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 345 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ser Lys Ser Gly Ala Leu Asp Leu Ala Ser Gly Leu Gly Gly Lys
1 5 10 15

Ile Asn Lys Val Glu Val Lys Ser Ala Val Asp Glu Tyr Glu Lys Tyr
20 25 30

His Gly Tyr Tyr Gly Gly Lys Glu Glu Ala Arg Lys Ser Asn Tyr Thr
35 40 45

Asp Met Val Asn Lys Tyr Tyr Asp Leu Ala Thr Ser Phe Tyr Glu Tyr
50 55 60

Gly Trp Gly Glu Ser Phe His Phe Ala His Arg Trp Asn Gly Glu Ser
65 70 75 80

Leu Arg Glu Ser Ile Lys Arg His Glu His Phe Leu Ala Leu Gln Leu
85 90 95

Gly Leu Lys Pro Gly Met Lys Val Leu Asp Val Gly Cys Gly Ile Gly
100 105 110

Gly Pro Leu Arg Glu Ile Ala Arg Phe Ser Ser Thr Ser Val Thr Gly
115 120 125

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Leu Asn Asn Asn Glu Tyr Gln Ile Thr Arg Gly Lys Glu Leu Asn Arg
    130                 135                 140

Leu Ala Gly Ile Ser Gly Thr Cys Asp Phe Val Lys Ala Asp Phe Met
145                 150                 155                 160

Lys Met Pro Phe Asp Asp Asn Thr Phe Asp Ala Val Tyr Ala Ile Glu
                165                 170                 175

Ala Thr Cys His Ala Pro Asp Pro Val Gly Cys Tyr Lys Glu Ile Tyr
            180                 185                 190

Arg Val Leu Lys Pro Gly Gln Cys Phe Ala Val Tyr Glu Trp Cys Ile
        195                 200                 205

Thr Asp His Tyr Asp Pro Asn Asn Ala Thr His Lys Arg Ile Lys Asp
    210                 215                 220

Glu Ile Glu Leu Gly Asn Gly Leu Pro Asp Ile Arg Ser Thr Arg Gln
225                 230                 235                 240

Cys Leu Gln Ala Val Lys Asp Ala Gly Phe Glu Val Val Trp Asp Lys
                245                 250                 255

Asp Leu Ala Glu Asp Ser Pro Leu Pro Trp Tyr Leu Pro Leu Asp Pro
            260                 265                 270

Ser Arg Phe Ser Leu Ser Ser Phe Arg Leu Thr Ser Val Gly Arg Met
        275                 280                 285

Ile Thr Arg Thr Met Val Lys Ala Leu Glu Tyr Val Gly Leu Ala Pro
    290                 295                 300

Gln Gly Ser Glu Arg Val Ser Asn Phe Leu Glu Lys Ala Ala Glu Gly
305                 310                 315                 320

Leu Val Glu Gly Gly Lys Lys Glu Ile Phe Thr Pro Met Tyr Phe Phe
                325                 330                 335

Leu Val Arg Lys Pro Leu Leu Glu  *
            340                 345
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